To study expression and assembly of TRPM1 channels we cloned seven full lengths TRPM1 cDNAs from mouse eye which represent splice variants and encode proteins of 1462, 1506, 1512, 1578, 1584, 1622 and 1628 amino acid residues. These TRPM1 protein variants vary in their N terminus, in a putative extracellular loop and the predicted pore region. For expression analyses we prepared polyclonal and monoclonal antibodies directed against various antigenic epitopes of TRPM1. All antibodies detect proteins of the size expected for TRPM1 in the eye, in lung and skeletal muscle. They immunoprecipitate the TRPM1 protein from mouse retina and specifically label postsynaptic dendrites of bipolar cells adjacent to the ribbon synapse. All TRPM1 proteins contain a coiled-coil domain in their C termini and isolated fragments comprising this coiled-coil domain have been shown to form dimers (1). We show that after disruption of this coiled-coil domain by site directed mutagenesis the full-length TRPM1 proteins still assemble to dimers and higher molecular protein complexes and interact with each other, apparently by additional intermolecular interactions of TRPM1 sequences within the N-terminus. Similarly, a related TRPM protein, TRPM4, still forms dimers after destruction of a C-terminal coiled-coil domain, most probably via interactions of N-terminal protein sequences.

1 Tsuruda PR, Julius D, Minor DL Jr (2007) Neuron 51, 201-212.

1789-Pos

A Reduction of Glucose-Induced Bursting Frequency in Pancreatic Islets Correlates with Decreased Insulin Release and Impaired Glucose Tolerance in TRPM5-/- Mice

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Glucose homeostasis is critically dependent on insulin release from pancreatic beta cells, which is strictly regulated by glucose-induced simultaneously oscillations in membrane potential (V_m) and cytosolic calcium concentrations $[Ca^{2+}]_{cyt}$. We propose that TRPM5, a Ca^{2+}-activated monovalent cation channel, is a positive regulator of glucose-induced insulin release. Micro-array screening and immunostaining reveal high and selective expression of TRPM5 in pancreatic islets. Whole cell current measurements in WT pancreatic islet cells demonstrate a Ca^{2+}-activated non-selective cation current with properties comparable to TRPM5 measured in over-expression, including the bell-shaped dependency on intracellular Ca^{2+}, time constant of activation and permeation properties. This current is significantly reduced in *Trpm5*$^{-/-}$ cells. Ca^{2+}-imaging and electrophysiological analysis show that glucose-induced oscillations of V_m and $[Ca^{2+}]_{cyt}$ have a reduced frequency in *Trpm5*$^{-/-}$ islets. WT islets display either slow or fast oscillations, or mixed oscillations, consisting of fast oscillations superimposed on slow ones. In contrast, *Trpm5*$^{-/-}$ islets never show a fast oscillation pattern. Fast oscillations in V_m show a shorter burst interval, due to a higher slope of depolarization towards the threshold potential for burst initiation. Our results indicate that TRPM5 accelerates the depolarization during the interburst interval, initiating rapid oscillations and higher insulin release. As a consequence, glucose-induced insulin release from *Trpm5*$^{-/-}$ pancreatic isletsis significantly reduced, resulting in an impaired glucose tolerance in these mice. Pharmacological modulation of TRPM5 activity may represent a novel means to adjust insulin release in diabetic patients.

1790-Pos

Physiological Role of the Oxidative Stress-Sensitive TRPM2 Ca^{2+} Channel in Immunocytes

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It is known that a large amount of reactive oxygen species (ROS) exist at inflamed sites. ROS induce chemokines responsible for the recruitment of inflammatory cells at inflamed sites. Here, we demonstrate that the plasma membrane Ca^{2+}-permeable channel TRPM2 controls ROS-induced chemokine production in monocytes/macrophages. In monocytes from *Trpm2*-defecient mice, H_2O_2-induced Ca^{2+} influx and production of the macrophage inflammatory protein-2 (CXCL2), which exhibit potent neutrophil chemotactic activity, were impaired. In the inflammation model dextran sulfate sodium-induced colitis, CXCL2 expression was attenuated by *Trpm2* disruption. Interestingly, the number of recruited neutrophils was significantly reduced in DSS-treated *TRPM2* KO mice, whereas that of DSS-induced macrophages after infiltration into inflamed sites, was indistinguishable in WT and *TRPM2* KO mice. Importantly, TRPM2 deficiency failed to impair important aspects of CXCL2-evoked neutrophil chemotaxis, including Ca^{2+} response, *in vitro* migration, and *in vivo* infiltration after DSS administration. Thus, TRPM2-mediated Ca^{2+} influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. We propose functional inhibition of TRPM2 channels as a new therapeutic strategy for treating inflammatory diseases.

1791-Pos

Multi-Dimensional Characterization of the Desensitization Behavior of Temperature- and H+-Activated Human TRPV1 Channels using Microfluidics

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This work describes the pH- and temperature-dependence of acute desensitization and tachyphylaxis in human TRPV1 channels. We use an in-house developed microfluidic device and associated methods, which independently can control the temperature and the solution environment (e.g. the pH) around patch-clamped cells, as well as the time a cell is exposed to different solutions. Thus, cells can be stimulated and controlled in a multi-dimensional parameter space. Our results show that if TRPV1 channels are exposed repeatedly to low pH applications, the rate of desensitization becomes progressively slower, whereas the rate of channel activation remains unchanged. Also, both the rate of activation and the rate of acute desensitization increase at higher temperatures. The extent of tachyphylaxis is found to be dependent on pH, temperature, and exposure time. We also show that both the desensitization rate and the extent of tachyphylaxis are correlated to current density. This could be due to the fact that Ca^{2+} is an important factor for both acute desensitization and tachyphylaxis, and since TRPV1 is permeable to Ca^{2+}, the current density is proportional to Ca^{2+} influx.

1792-Pos

A Novel Tarantula Toxin Irreversibly Activates TRPV1

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Spider venoms contain an evolutionarily honed pharmacopeia of natural toxins that target membrane receptors and ion channels to produce shock, paralysis, pain, or death. Toxins evolve to interact with functionally important protein domains, including agonist binding sites, ion permeation pores, and voltage-sensing domains, making them invaluable reagents with which to probe mechanisms underlying channel activation or modulation. We have identified a novel tarantula peptide toxin that serves as an irreversible agonist for the heat/capsaicin-activated channel, TRPV1. The toxin contains two independently functional Inhibitor Cysteine Knot (ICK) domains, endowing it with an antibody-like bivalency that results in extremely high avidity for its multimeric channel target. We are using this new toxin as a tool to help dissect the unique properties of TRPV1.

Muscle: Fiber & Molecular Mechanics & Structure II

1793-Pos

Passive Force Augmentation in Actively Stretched Myofibrils and Sarcomeres

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Single skeletal muscle myofibrils were stretched actively and passively ($pCa^{2+} = 3.5$ and 8 respectively) from optimal length to beyond myofilament overlap (4.0μm) and individual sarcomere lengths and the associated stretch forces were measured. Actively stretched myofibrils produced much more force than passively stretched myofibrils at all sarcomere lengths, including when all sarcomeres were beyond myofilament overlap (Figure 1). In order to confirm that cross bridge based acto-myosin forces were not present at sarcomere lengths beyond myofilament overlap, passively and actively stretched myofibrils were deactivated and activated, respectively at 5μm. In both cases,

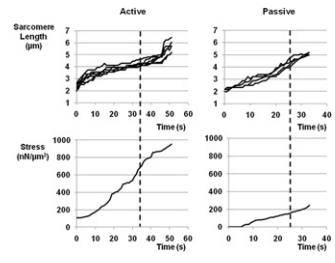

Figure 1. Individual sarcomere length and force traces for single active and passive myofibrils. Actively stretched myofibrils show much greater force than passively stretched myofibrils at sarcomere lengths beyond myofilament overlap where actin-myosin based cross-bridge forces are zero. Dashed vertical line denotes when all sarcomeres are beyond myofilament overlap.